I.1 How to handle biological specimens

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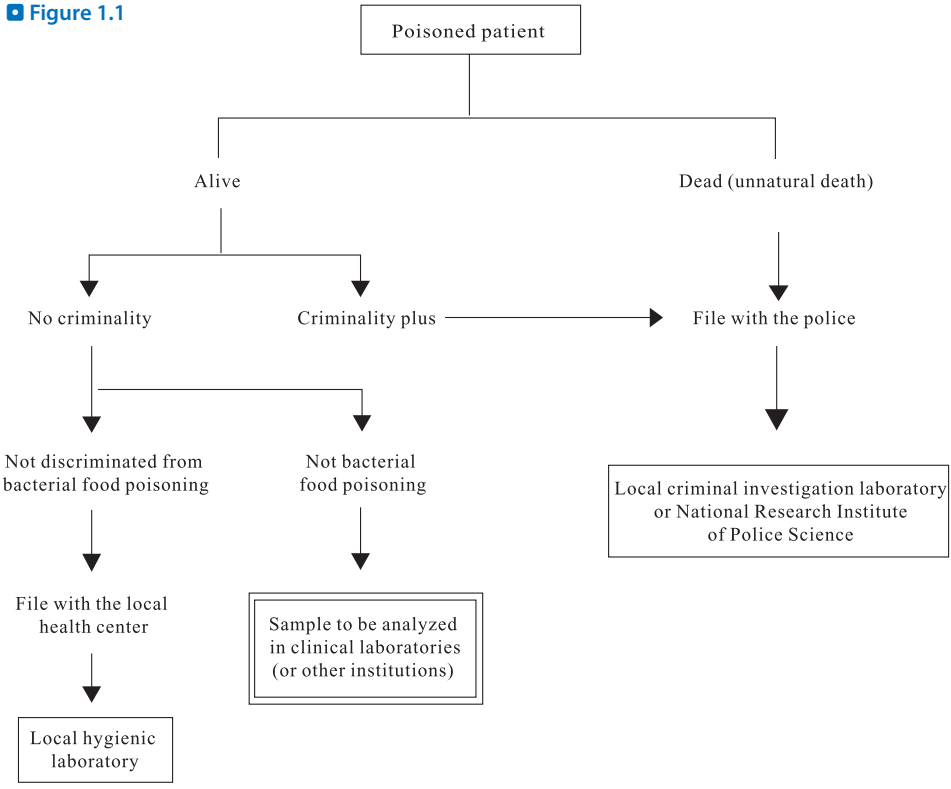
Some knowledge to be required before handling specimens

The flowchart for how to handle specimens obtained from poisoned patients is shown in *Figure 1.1*. When a poisoning incident takes place and a patient is sent to hospital, medical doctors and co-medical staffs should concentrate their efforts on the intensive care of the patient. However, at this point, the discrimination whether it is a poisoning case or not is, of course, very important. If the patient dies and the death is judged due to poisoning, the responsible doctor should file the death with the police located at the district within 24 h according to a law in Japan.

Figure 1.1



Flowchart for how to handle specimens obtained from poisoned patients.

The death due to bacterial food poisoning should be classified into intrinsic one (disease death), and be discriminated from the death due to drugs or poisons (extrinsic death); it is not necessary to file with the police, but it should be filed with a local health center. Irrespective of being dead or alive of the patient, both bacterial food poisoning and drug poisoning should be filed with a local health center. It should be mentioned here that deaths due to ingestion of puffer fish and mushrooms are classified as extrinsic ones.

After the police accepts the file of an unnatural death, the analysis of a causative toxin is made, according to the need, at a local criminal investigation laboratory of police headquarters in each prefecture in Japan. When the analysis at the local laboratory is difficult, the specimens are sent for analysis to the National Research Institute of Police Science in Kashiwa City, Chiba Prefecture. When a cadaver of unnatural death is subjected to judicial autopsy at a department of legal medicine of a medical school, the toxin specimens obtained from the cadaver is analyzed at the department in case that the department is capable to analyze it.

When a patient survives and criminality is suspected in a poisoning case, it should be filed with the police immediately; in such a case, the police is absolutely responsible for the toxin analysis.

Only when a poisoning patient is alive with no criminality, and also no discrimination between poisonings by bacterial food and by drugs/poisons can be made, a request addressed to a local hygienic laboratory for toxin analysis is possible *via* a health center. The main duties of the hygienic laboratory are bacterial tests on foods and analysis of environmental pollutants; the laboratory is usually equipped with expensive analytical instruments such as mass spectrometers, and seems to sufficiently meet the analysis of drugs and poisons. However, at the present time in Japan, such request is usually rejected by the laboratory after the possibility of being bacterial food poisoning is excluded. Therefore, a problem arises concerning which institution undertakes the analysis of specimens collected from a poisoning patient admitting at a hospital, who survives and shows no criminality (suicide trial or accident). The best way is that the clinical laboratory of the same hospital undertakes the analysis of the specimens; however the analysis of drugs and poisons is almost impossible at a local hospital, because it is usually not easy, and requires a skill of analysts. Unfortunately, in Japan, the so-called poison control centers for undertaking the toxin analysis are not available; while in the US and Europe the poison control centers are active for analysis of such specimens. Our problem is not due to the scientific delay in analytical chemistry in our country, but is due to the delay in measures to be taken by Japanese Government. To overcome the above problem, much efforts are being made at non-governmental levels [1]; one of the efforts is presented in Chapter I.7.

Sampling of specimens on a clinical scene

Blood

Blood specimens are now being collected from the vein using vacuum sampling tubes; EDTA, citrate or heparin is usually contained in the tube as an anticoagulant. Some tubes contain sodium fluoride as a preservative. The analysts should be aware of the presence of such additives.

Larger amounts of blood are preferable to be sampled for toxin analysis; however, in view of the stress to patients, 5–10 mL of blood is to be sampled. If a situation permits, multiple samplings at different intervals are desirable. The time-course analysis is very useful for deciding the therapeutic policy in poisoning cases. When plasma is required, the supernatant fraction is obtained by centrifuging the tubes containing whole blood at 2,000–3,000 rpm.

Urine

Also for urine, larger amounts are preferable to be sampled. When urine is obtained by catheterization from a patient, it should be taken into mind that a jelly containing a local anaesthetic had been applied to the catheter; urine is usually contaminated by such a drug. Also for urine, the samplings according to time intervals are preferable. According to the need, sodium fluoride or sodium azide is added to urine samples at a concentration of 1 mg/mL as a preservative.

According to the kinds of drugs and poisons, large amounts of metabolites are sometimes excreted into urine. Before analysis, some knowledge on the metabolism and excretion for a possible toxin is needed; a useful dictionary was published for such a purpose [2].

Vomitus and gastrolavage fluid

After oral ingestion of a drug or a poison, there is a possibility that gastric contents contain a high level of an unchanged toxin. The vomitus and gastrolavage fluid should be stored in amounts as large as possible; their volumes should be strictly recorded. Also according to the need, sodium fluoride or sodium azide can be added as a preservative.

Hair and nails

When chronic intoxication by a drug or a poison (especially heavy metals and basic drugs) is suspected, after getting the consent from the patient, several pieces of long hair are sampled by cutting off at their roots, put in a dry polyethylene bag with a fastener and kept at room temperature or 4° C.

Nails are also good materials for detection of a drug or a poison which was ingested in the past, and can be an alternative specimen, especially when the scalp hair is too short or not available. They can be kept also at room temperature or 4° C.

Collection of informations on a possible drug or a poison administered or ingested

Inquiries on a drug or a poison to the patient and his/her family are essential. Efforts should be made to find a cup or a bottle left on a poisoning spot, because there is a possibility that pure or clean solution of a toxic compound is contained in it. It is a good method to ask members of the emergency services to look for such items on the spot.

Sampling from cadavers

Observation on the spot of poisoning

When a medical doctor is requested to make postmortem inspection, he/she should arrive at the spot of poisoning to achieve the duty. Before the inspection of a cadaver, the doctor should observe the surrounding situations as carefully as possible, and also should sniff the air. There are many fatal cases of organophosphorous pesticide and cresol poisonings, in which a strong aromatic smell is given from the vomitus and from the mouth of a cadaver. When no finding of vomiting is observed, it is sometime useful for the doctor to try to sniff the smell by keeping doctor's nose closer to the nose and mouth of the cadaver and by pushing its chest slowly. Many of cadavers due to poisoning show dirty mucous fluids, froth or degeneration of the lip or around the mouth. It is also essential to look for a bottle or a cup containing a toxic compound. When they are found, they should be carefully stored until analysis. The packages and plastic cases for tablets and capsules should be looked for especially in a trash can or other places in the room. If vacant packages or cases are found in the trash can, they should be carefully lined up according to upper-to-lower layers. In case of failure to find out them inside the room, such search should be extended to nearby places, where trash is gathered outdoors.

When it is disclosed that the victim has visited a clinic or a hospital, detailed informations can be obtained on the kinds of drugs and their amounts prescribed; informations on the diagnosis of diseases and the time of the last visit can be also obtained by making inquiries to the responsible doctor. On every package or case for tablets or capsules, code numbers or special marks are usually shown; it is easy to identify a drug by the code numbers or marks using a drug-list book [3]. In most cases of poisoning, victims usually ingest multiple kinds and large amounts of drugs to commit suicide. The final judgement whether a death is due to drug poisoning should be made by counting the number of drugs and by considering the toxicity of each drug ingested.

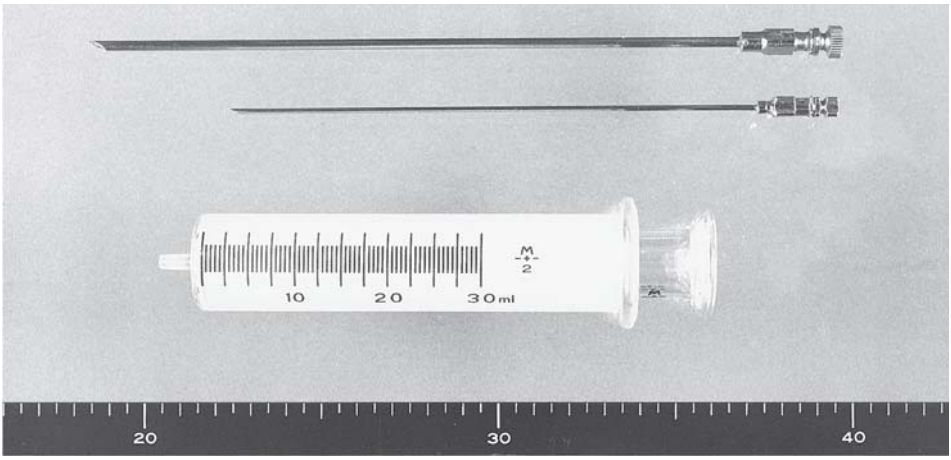
Sampling at postmortem inspection

When vomitus and gastrolavage fluid are available, it is preferable to keep all of them. Since the froth or saliva fluids attached to the lips or their surrounding skin may contain high concentrations of a drug or a poison, they should be sampled by wiping them carefully with gauze and be kept in a sealed case at room temperature or 4° C.

The author et al. usually sample about 10 mL blood at every postmortem inspection, even if the cause of death is strongly suggested to be only disease; we keep it at –80° C for at least 1 year. This is because any unpredictable matters may be disclosed by further investigation of the police. Especially in rural areas without a medical examiner system (this system is active only in some big cities in Japan), the cadavers, which are considered not involved in criminality, are usually not autopsied, but subjected only to postmortem inspection. Therefore, the storage of a blood sample for a long time seems very important, because there is a possibility that the sample will serve as an effective evidence in the future.

Since the punctures for samplings are based on the request of a judicial police officer, they are not illegal; but it is preferable to get the consent of family members upon samplings.

Figure 1.2



Needles and a glass syringe for punctures. The upper big needle is used for stomach puncture; the smaller one for cardiac, suboccipital and urinary bladder punctures.

The needle usually used for lumbar puncture can be used at postmortem inspection. However, we are using so-called "a contrast medium needle", which is thicker and longer than that for lumbar puncture (*Figure 1.2*); it is about 16 cm long and its internal diameter is about 1 mm; it is useful for cardiac, urinary bladder and suboccipital punctures. For stomach puncture, we are using even thicker and longer needles (20 cm long and 1.5 mm internal diameter) (*Figure 1.2*), because the stomach usually contains solid contents. The marking with an oil-based marker pen at the sites of 5 and 10 cm from the tip of a needle is useful to estimate the depth of puncture. A conventional glass syringe of a 10–30 mL volume is recommendable rather than a plastic disposable syringe, because the glass syringe easily gives subtle touch sense to be transmitted to the finger upon drawing blood.

For cardiac puncture, the needle should be stuck rapidly at the depth of about 10 cm on the following location of the chest; on the straight line combining both nipples, on an intercostal space and at the left margin of the sternum. After removing the inner needle, the glass syringe is connected to the needle; together with pulling the plunger, the needle position is moved back and forth slowly. When blood is present in the heart, it is easily withdrawn into the syringe; at least 5 mL blood is sampled and stored.

For urine sampling, the pubic symphysis is palpated, and the needle is stuck into the urinary bladder at the upper margin of the pubic bone at an angle of about 45 degree against the abdominal skin surface. When a large amount of urine is present in the bladder, it is easily withdrawn into a syringe. Of course, the sampling of urine by catheterization *via* the urethra is possible like in the case of a living patient. Larger amounts of urine are preferable for the case in which poisoning is suspected.

For sampling of stomach contents, the above large needle is rapidly stabbed toward the stomach at the inner margin of the left costal cartilages. When a large amount of stomach contents is present, it is easily obtainable. However, it is not easy when their amount is small; it is difficult to inject the needle through the stomach wall, because the latter is too movable in the absence of a large amount of stomach contents.

According to the need, cerebrospinal fluid (CSF), hair and nails are sampled. CSF is sampled by suboccipital puncture as follows. The neck is bent forward, and the needle is stuck from the backside at the level between the foramen magnum and the first jugular vertebra to reach the cisterna magna; more than 10 mL of CSF can be obtained by such puncture. As stated before, there are many cases in which basic drugs or poisons are relatively stably retained in hair or nails for a long time ; hair and nails sometimes become good alternative specimens for analysis of drugs and poisons in putrefied cadavers, and may be also useful for detection of toxins which had been taken or administered in the past. The utility of hair and nails is presented in Chapter I.2 entitled "Alternative specimens" of this book.

Puncture needles should be kept clean; after their use, blood attached to the needles should be immediately removed by washing with tap water by moving the inner needles back and forth. The bloody needles should not be left to dryness after use.

Sampling at autopsy

When death by poisoning is suspected, stomach contents, right and left heart blood and urine are collected as much as possible (10–100 mL) and stored. More than 20 g of each tissue of the brain, lung, heart, liver, kidney and spleen should be sampled. In case of a putrefied cadaver, the skeletal muscle in the thigh may become a useful specimen for analysis, because the tissue of this part is most resistant to putrefaction and contains levels of drugs and poisons almost equal to those in blood. The tissues from different organs should not be put in the same container or polyethylene bag; they should be kept separately. Special care should be taken for the stomach contents, because it may contain a very large amount of a drug or a poison, which can contaminate other specimens.

When subcutaneous or intramuscular injection of toxins is suspected, the probable injection site is incised, and the skin is carefully removed to sample the corresponding subcutaneous adipose tissue or muscle, which may contain high levels of unchanged drugs or poisons.

Storage of samples

Blood or urine obtained from a living patient or a cadaver is kept in a glass vial (or tube) with a Teflon screw cap; the vial should be sealed completely. When a polyethylene or plastic tube is used, the contamination of the sample by a plasticizer and other compounds should be taken into mind. Solid samples (organs and tissues) are separately put in small polyethylene bags with fasteners to prevent them from drying. It is preferable that every sample is prepared in duplicate; one is kept at 4° C for analysis within a few days and the other kept at –80° C for a long storage. When glass tubes are kept at temperatures below 0° C, the rupture of glassware due to expansion of frozen fluids should be avoided by leaning or laying the tubes.

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